Study the effect of cerium oxide nanoparticles on corneal injury healing in rabbits

Abstract

Background: Injury in the cornea in animals tends to heal slowly due to a vascular structure. Therefore, treatments that accelerate the healing of the cornea need to be investigated. **Object**ive: To enhance and evaluate the healing process of corneal injury using clinical assessments and fluorescent dye examinations. **Materials & methods:** Thirty-six rabbits (Britannia Petite) were used in this study. Under general an aesthesia by injection of (10 mg/kg) xylazine and injection of (25 mg/kg) ketamine HCl, corneal injury was induced with NaOH 0.4%, applied to the cornea of the right eye for 20 seconds. The animals were divided into three groups: group one (control group, 12 rabbits), group two (treated group, 12 rabbits), and group three (treated group, 12 rabbits). The animals were examined clinically during the studied period, and fluorescence dye evaluation was performed at the 1st, 3rd, and 4th weeks post-operation. **Results:** After 28 days of inducing a corneal injury, the control group still had a fibrous tissue layer and corneal opacity on the corneal surface. However, corneal opacity exhibited greater transparency. While, in the treated group, we will observe the complete healing of the eye, with the restoration of blood and nutrient supply to the cornea, resulting in the total resolution of all corneal damage. **Conclusion:** The results of this study demonstrated the Nano cerium oxide acceleration and enhanced of the corneal wound healing because have anti-inflammatory, antibacterial, and antioxidant properties.

Keywords: Nanoparticles, Cerium Oxide, Cornea Anatomy. Corneal injuries

Introduction

Corneal injuries can occur from foreign objects; improperly fitted contact lenses; bacterial or viral infections; bleach, acid or alkaline chemicals; and exposure to UV rays, tanning beds, or UV reflections, causing temporary or permanent damage and vision loss. Ulcers may be located centrally, paracentrally, or peripherally and can be superficial or deep (Gurnani *et al.,* 2023). The main factors influencing corneal epithelium repair post-injury include the corneal stem cell pool, vascular supply and early inflammation, and corneal nerve endings, as well as electrochemical gradients, extracellular matrix, and cellular communication. The treatment of corneal ulcers requires a multifaceted approach due to the avascular nature of the normal cornea. Medications are utilised to avert the bacterial infections (the ophthalmic antibiotic drops or ointment) and to alleviate spasms and the pain (ophthalmic atropine drops or ointment) (Mohan et al., 2023; Jasim et al.,2025). Certain situations may necessitate surgical intervention, particularly in instances of imminent corneal perforation or when medicinal treatment is ineffective (Deshmukh *et al.,* 2020).

Surgical intervention may be required for about 30 to 40% of the corneal ulcers, depending upon the severity of infection at presentation. Criteria for surgical intervention in the infectious keratitis encompass non-healing or unresponsive ulcers, the ulcers that deteriorate despite medicinal treatment, progressive corneal thinning, descemetocele formation, and eventual corneal perforation. In critical instances, a corneal ulcer can lead to the corneal transplant. A corneal transplant entails the surgical excision of corneal tissue, succeeded by the implantation of donor tissue. (Tuli *et al.*, 2016). Cerium oxide nanoparticles demonstrate various biological characteristics, facilitating their widespread application in biomedical fields (Yaser *et al*., 2025). CeO_2 nanoparticles have demonstrated the ability to enhance wound healing by reducing inflammation, decreasing oxidative stress responses, reducing infection risks, and promoting angiogenesis during the healing process. These characteristics make CeO_2 nanoparticles an attractive contender for wound healing applications (Walkey *et al.*, 2015; Yasser *et al.*, 2025). CeO_2 nanoparticles can both facilitate and inhibit angiogenesis (Cheng *et al.*, 2021).

Materials and Methods:

Animals

Thirty-six adult rabbits which are a year old and weigh about 1.5±0.5 kg were purchased. All animals had free access to the diet and drinking water and were maintained at the temperature of the room (Hashim & Nazht, 2021).

Experimental design

Thirty-six adult rabbits were used in this study. All the rabbits are divided into three groups, each group containing 12 rabbits randomly; Group one (Control group) (n=12), the animals received distal water topical drops one-day post-injury daily and persisted for seven days. The group two (treated group) (n=12) were the animals treated by cerium oxide, which is locally applied at the injury site of the cornea daily with a concentration of 5 µg/ml for one week. The group three (treated group) (n=12) were the animals treated by cerium oxide, which is locally applied at the injury site of the cornea daily with a concentration of 10 µg/ml for one week.

preparation of cerium oxide nanoparticle

Nanoparticle cerium oxide (10-30 nm) in size was prepared and diluted as follows according to Malyla (2023):

To prepare cerium oxide nanoparticle at a concentration of 5 µg/ml, take 0.5 mg of cerium oxide nanoparticle, which is diluted with 100 ml of distal water, then mixed well in a flask, and then each (1) ml contains (5 µg/ml) of cerium oxide nanoparticle.

And prepare cerium oxide nanoparticles at a concentration of 10 µg/ml; take 1 mg of cerium oxide nanoparticles that were diluted with 100 ml of distal water, and then each 1 ml contained 10 µg/ml of cerium oxide nanoparticles. This material was prepared at the Research Centre in the College of Veterinary Medicine.

preparation of NaOH

NaOH is prepared according to the following formula (Mane, 2011):

100×volume of the solution in (ml) /W/V% = weight of the solute in (g)

Dissolve (4g) of NaOH in 100 ml of distal water at the research Centre in the College of Veterinary Medicine and close the container tightly.

Method of Inducing Corneal Injuries

Rabbits were anaesthetised by intramuscular injection of (10 mg/kg) xylazine and injection of (25 mg/kg) ketamine HCl (Hashim *et al.*, 2021; and Akter *et al*., 2023). The experiment was carried out on the right eye of each of the animals. The body temperature was maintained at a temperature between 37 and 37.5°C. Corneal wound was performed by alkaline burn as described by (Gronkiewicz *et al.* 2016). A round (10 mm) diameter circular filter paper disc soaked in 4% sodium hydroxide (NaOH; 4 µl, 1 mol/l) and applied to the cornea of the right eye for 20 seconds, then the disc was removed and the cornea rinsed with sterile distal water for 1 minute (figure 1).

Parameters of study

1. **Evaluation in the clinic**

Complete external and complete ophthalmic examinations of both eyes have been conducted. The eyes have been examined for corneal ulcers, eyelid adhesion, lacrimation, infection, or purulence. The healing process of the ocular surface for each treated eye was assessed and evaluated at 1, 2, 3, and 4 weeks' post-treatment.

2. Fluorescence dye examination

A piece of blotting paper infused with fluorescent dye will be applied to the surface of the rabbit's eye. The rabbit will blink. Blinking disperses the dye, enveloping the "tear film" that covers the corneal surface. A blue light is subsequently aimed at the rabbit's eye using an ophthalmoscope. The experimental corneal ulcer will be dyed and have a green hue under blue light.

Statistical analysis

Standard errors were incorporated into the results. A statistical software tool (SPSS for Windows version 22, USA) was utilised to conduct one-way ANOVA with multiple comparison tests on data. The significance threshold for the differences was established at ($P \leq 0.05$) (Abdulrazak *et al*., 2018).

Results

1- Clinic evaluation

The results of the clinical findings show that after the induction of corneal injury in all groups, the freshly burnt corneal tissue becomes hazy immediately after the burn and subsequently turns opaque within 24 hours. That appears to be a sharp damage in the stromal layer of the cornea. The animal appears to have clinical signs of opacity, lacrimation, itching, and discharge (Figure 2, A).

In the first 3 days, rabbits exhibited blepharospasm as a result of pain associated with ulcer formation, leading to semi-closed eyes. In the first week, corneal ooedema was the most prominent sign after induced corneal injury in all groups, and we noticed conjunctival ooedema, swelling of the eyelid, and the appearance of clinical signs in the animals, such as photophobia, eyelid adhesion, signs of conjunctivitis, purulent exudates, and obvious corneal ulcers. On the seventh day of the treatment group, we observe a decrease in the severity of the inflammatory signs, but the swelling and corneal opacity remain (figure 2, B).

The second week after inducing corneal injury, the control group exhibited persistent eye swelling, discharge, eyelid swelling, corneal opacity, purulent exudates and inflammatory signs (Figure 3, A).**whereas in the treatment group** (5µg/ml cerium oxide nanoparticle) showed resolution of inflammatory signs, although corneal opacity persisted, tear production diminished, the capillaries, which are very small blood

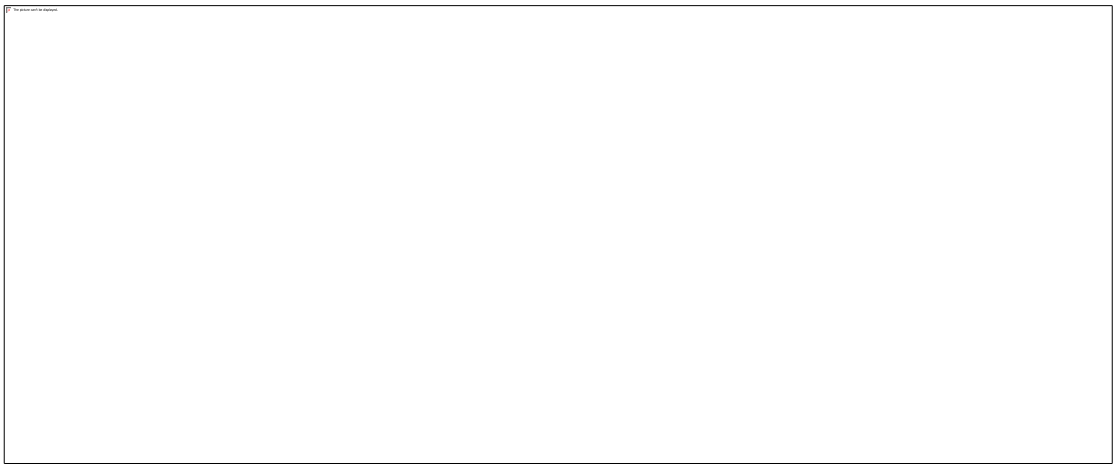
vessels, grew, and minimal swelling remained with the cessation of eye discharge (Figure 3B).

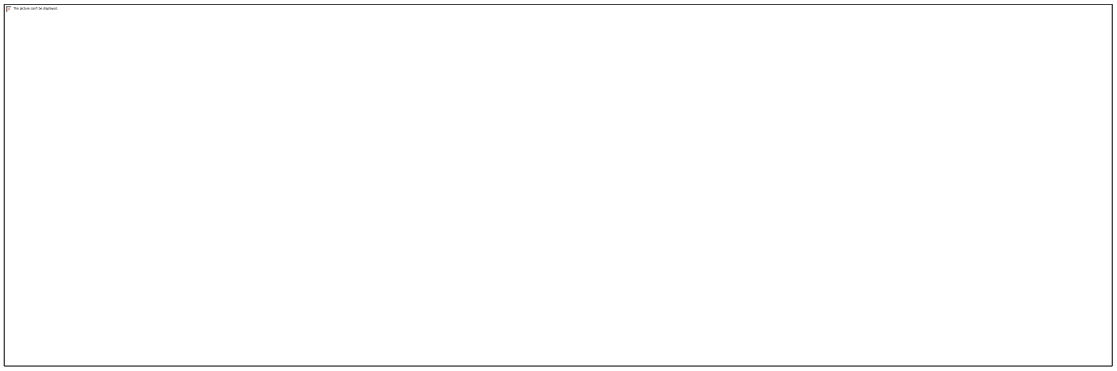
In the treated group (10µg/ml of cerium oxide nanoparticle), all inflammatory signs will resolve, the corneal opacity will decrease, the blood vessels will grow more clearly visible near the cornea, and the oedema will disappear (Figure 3, C).

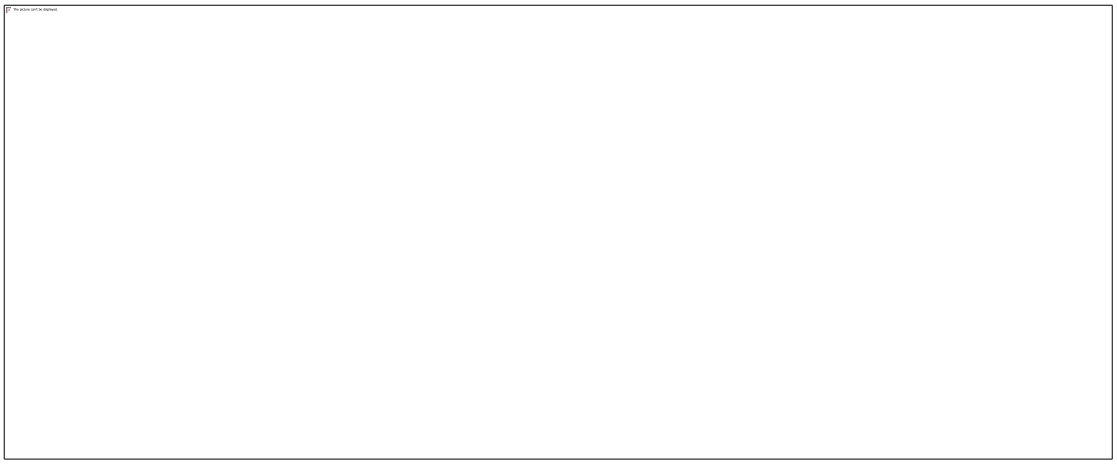
Three weeks after inducing corneal injury in the control group, all signs of inflammation disappeared, leaving only corneal opacity. A little redness remained, purulent secretions reduced, and the eye ulcer became small in shape and size (Figure 4, A). **However, in the treated group** (5µg/ml of cerium oxide nanoparticle), the corneal ulcer was transparent and smaller in size, and new blood vessels developed at the location of the corneal injury and were clearly visible (Figure 4, B).

In the treated group (cerium oxide nanoparticle), the size of the corneal ulcer is small, and there are numerous blood vessels around the ulcer. Additionally, we only observe a small scar in the centre of the cornea figure (4, C).

In the fourth week after inducing a corneal injury in the control group, a fibrous tissue layer persisted on the corneal surface, and the corneal opacity persisted. However, the eye opacity is more transparent than that of the control group during the initial phases of the study and small in size and shape (Figure 5, A). **Whereas in the treated group** (5µg/ml of cerium oxide nanoparticle), all inflammatory signs disappeared – the swelling and redness – and the eye returned to its normal state, (table 1). **A treated group** (10µg/ml of cerium oxide nanoparticle) showed full restoration of the cornea, the eye returning to its normal state, and the resolution of all ocular issues. The rabbits in this group exhibit normal vision (Figure 5, B).







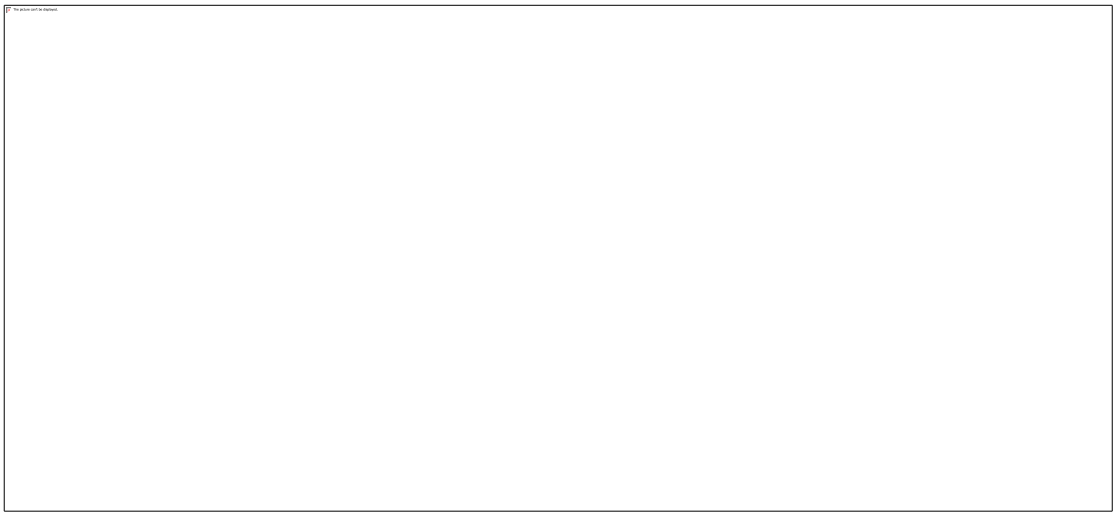
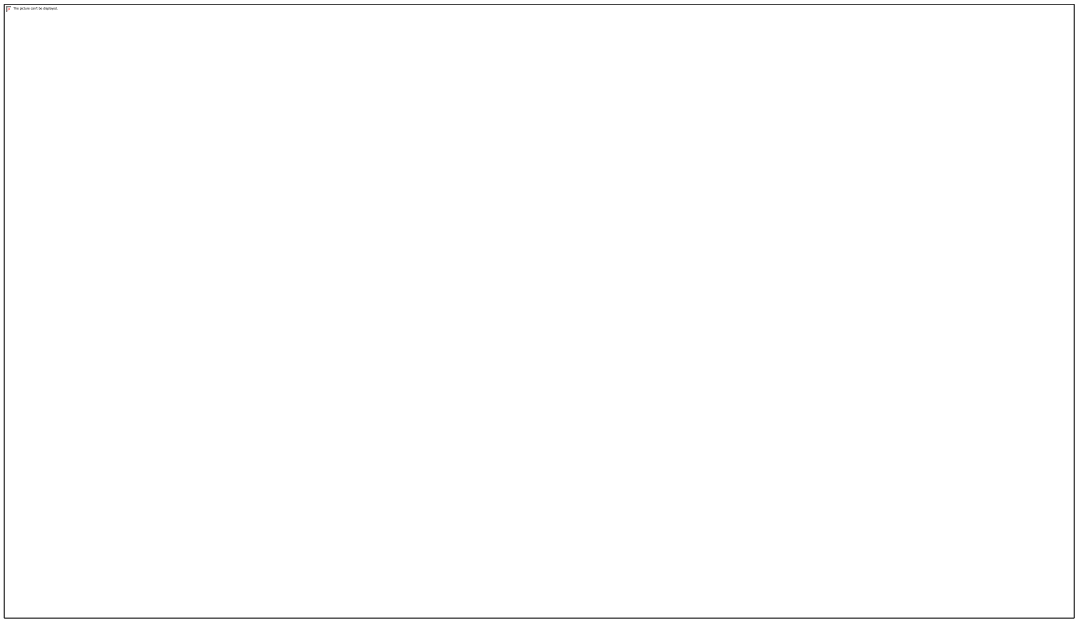


Table (1) shows the comparison of corneal opacity in all groups among the periods of study.



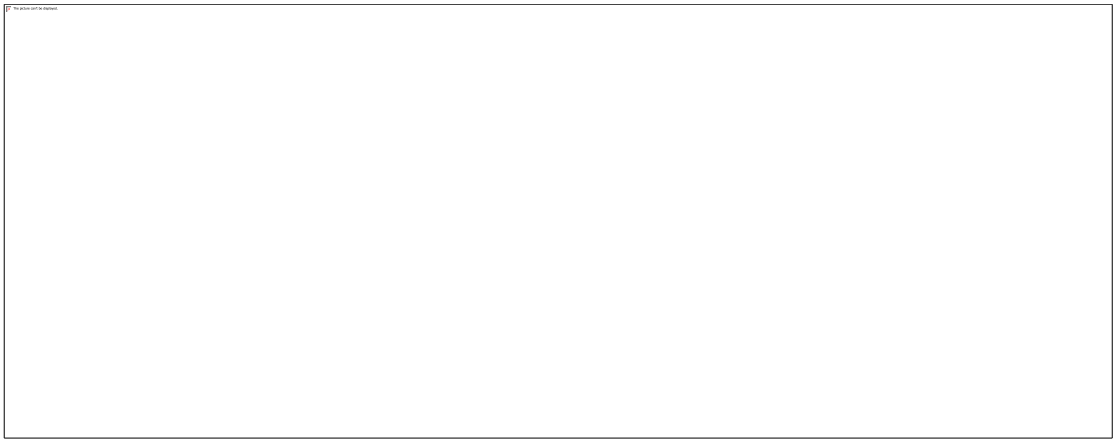
2. **Fluorescent staining**

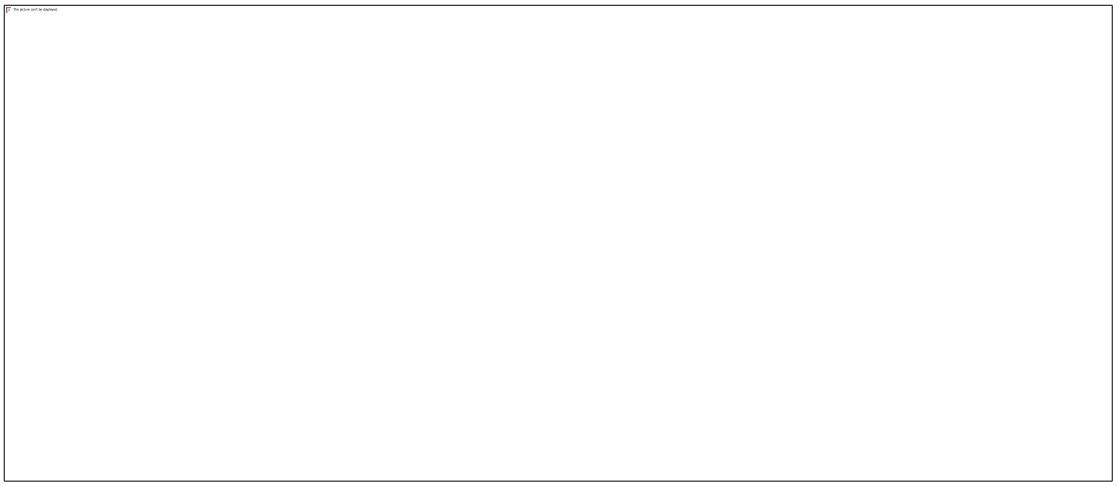
A healthy cell exhibits minimal permeability to fluorescein, whereas a compromised epithelium fails to obstruct fluorescein from infiltrating the damaged tissue and diffusing through the paracellular space into the underlying epithelial layers. Consequently, fluorescein does not dissipate with blinking and tear flow as readily as the healthy, smooth, and taut areas of the neighbouring cornea (Figure 6, A). A corneal ulcer is an open sore in the cornea of a rabbit. The corneal ulcer usually appears as a white or dull greyish lesion on the eye cornea. Occasionally, the ulcers form throughout the entire cornea and may extend deeper. The pus may accumulate behind the cornea, occasionally resulting in a white film at the bottom of the cornea. The conjunctiva is usually bloodshot (Figure 6, B).

Immediately in all groups after inducing the alkaline burn and using the fluorescein staining of the eye cornea, a corneal ulcer will appear as a yellow-green area of uptake using blue light on the ophthalmoscope device (Figure 6, C).

Following 21 days of inducing a corneal injury, in the control group a little redness remained, there was a reduction of purulent secretions, and the eye ulcer became small in shape and size figure (7 A). In the treated group a reduction in the ulcer's size and a decrease in the opacity of the eye will be observed, and the development of new blood vessels will be observed (Figure 7B).

Following 28 days of inducing a corneal injury in the control group, a fibrous tissue layer remained on the corneal surface, and corneal opacity remained. However, corneal opacity exhibited a greater transparency figure (8 A). The treated group will observe the complete healing of the eye, with the restoration of blood and nutrient supply to the cornea, resulting in the total resolution of all corneal damage (Figure 8B).





Discussions

This study indicated that cerium oxide nanoparticles enhanced corneal wound healing, restored functional corneal nerves, and protected corneal cells from oxidative stress. Treatment with cerium oxide nanoparticles promotes reinnervation of the damaged cornea in rabbit eyes. The study of cerium oxide nanoparticle (CeO_2) on the healing of the corneal wounds in rabbits explores potential therapeutic benefits of this nanomaterial for ocular injuries.

Previous studies of Nano-cerium oxide have shown promise in medical applications because of its antioxidant, anti-inflammatory, and regenerative properties, making it a candidate for promoting tissue healing (Sadidi *et al*.,2020). The cerium oxide exhibits strong redox activity, mimicking enzymes like superoxide dismutase (SOD) and catalase, which help neutralise the reactive oxygen species (ROS). ROS accumulation is a common issue in tissue damage, including corneal wounds, where oxidative stress can delay healing. By reducing oxidative stress in damaged corneal tissues, cerium oxide nanoparticles can accelerate healing by maintaining a balanced cellular environment. In the present study the rabbits have shown that applying CeO_2 nanoparticles to corneal wounds can reduce inflammation, leading to less scarring and better tissue regeneration compared to untreated wounds (Lord *et al*.,2021). Previous

studies have shown the importance of cerium oxide nanoparticles in preserving ocular surface homeostasis, indicating potential protective benefits against injury (Yu *et al*.,2019). Cerium oxide nanoparticles are crucial for several corneal activities, aiding in the maintenance of corneal integrity and a sustaining epithelial barrier and cellular viability. The anti-inflammatory, antibacterial, and antioxidant properties of cerium oxide nanoparticles have been established (Cui *et al*.,2022).

Also, for the other results of this study, we used fluorescein dye to assess the ulcer's size and boundaries after the injury. We observed that the cornea absorbed the dye and changed its colour to yellow, indicating corneal injury and the formation of an opacity. In the third week, after examining the eye with fluorescein dye, we notice a slight reduction in the size of the ulcer in the control group. However, we observe a smaller and less opaque ulcer in the treatment group compared to the control group, and the eye absorbs less fluorescein dye in this group. But in the fourth week (at the end of the experiment), the ulcer disappears, the eye returns to normal, and the lack of dye absorption after the examination indicates complete healing. In the treatment group, after administering the dye, we notice the presence of small scars and a little opacity, and these scars will be stained with fluorescein after administration.

Prior research has demonstrated that the cornea is examined for epithelial abnormalities and subsequently assessed using fluorescein dye. Corneal epithelial defects exhibit a green hue when illuminated with cobalt blue light on the ophthalmoscope or slit lamp. The morphology and position of the epithelium defect may provide insights into the diagnosis (Kim, Jeehee. 2000). Traumatic defects may first have irregular edges, which subsequently repair from the periphery, resulting in a stellate healing pattern. Previous research has demonstrated that to visualize an ulcer distinctly, it is necessary to administer eye drops containing a yellow-green dye known as fluorescein. The fluorescein temporarily stains damaged regions of the cornea, enabling the visualization of areas that are normally imperceptible (Carr, N. J. 2022).

Conclusions

The study showed that cerium oxide nanoparticles can help repair the corneas of rabbits with cornea injuries by stimulating keratocyte activity. The high antioxidant capacity of cerium oxide nanoparticles contributes to faster healing in rabbit cornea injuries. Therefore, our results suggest the potential pharmaceutical use of cerium oxide nanoparticles for healing of the cornea injury.

Ethical Approval

All of the experimental procedures were performed in accordance with Basrah University, Veterinary Medicine College guidelines for the welfare of experimental animals. The animal experimentation ethics approval **(61/37/2025**).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies, such as large language models (ChatGPT, Copilot, etc.) and text-to-image generators, have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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